# Methicillin-Resistant *Staphylococcus aureus* in Wound Cultures Recovered From a Combat Support Hospital in Iraq

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**Background:** *Staphylococcus aureus* infections complicate care of combat-related injuries and can independently result in skin and soft-tissue infections during deployments or training. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains seem to produce severe disease but retain susceptibility to many oral antimicrobials. This study characterizes 84 MRSA isolates recovered from wound cultures at a combat support hospital in Iraq.

**Methods:** MRSA strains recovered from December 2007 through March 2009 were analyzed. Antimicrobial resistance testing was determined by broth microdilution and the BD Phoenix Automated Microbiology System. The genotypic pattern was analyzed by pulsed-field gel electrophoresis and polymerase chain reaction identification of resistance and virulence genes.

**Results:** No MRSA isolates from wound cultures were resistant to vancomycin. The most active oral antistaphylococcal agents were tetracycline (95% susceptibility), trimethoprim-sulfamethoxazole (94%), and clindamycin (94%). Of agents not typically recommended as monotherapy, 98% of isolates were susceptible to rifampin, 91% to moxifloxacin, and 60% to levofloxacin. The most common pulsed-field type (PFT) was USA300 (79%). The typical staphylococcal cassette chromosome *mec* IV elements carrying the CA-MRSA resistance genes were present in 88% of the isolates. Panton-Valentine leukocidin virulence genes were identified in 88% of isolates, including 100% of PFT USA300. The virulence gene associated with an arginine catabolic mobile element was present in 75% of isolates, including 94% of PFT USA300.

**Conclusion:** This study is the first genotypic and phenotypic characterization of CA-MRSA recovered from wound cultures in a deployed combat hospital. The pattern noted was similar to that seen in soldiers stationed in the United States.

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Staphylococci have been reported as major pathogens causing wound infections throughout 20th century US wars.[1–12] The resistance of *Staphylococcus* to penicillin was described soon after its introduction during World War II.[6] During the Vietnam War, *Staphylococcus* was commonly recovered in association with short- and long-term morbidity, and penicillin resistance was noted in up to 42% of isolates.[7–11] Casualties of Operation Iraqi Freedom and Operation Enduring Freedom continue to suffer from acute and chronic infections with *Staphylococcus aureus*.[13–15] This pathogen has been noted to be commonly recovered among patients being cared for in a combat support hospital (CSH) and colonizing wounds at the time of injury.[16,17]

Major concerns associated with managing *S. aureus* infections are increasing antimicrobial resistance and changing genotypes. Penicillin resistance was noted soon after its introduction into clinical care, and methicillin-resistant *S. aureus* (MRSA) emerged in the 1960s.[18–21] During the past two decades, an epidemiologic shift of MRSA infections has occurred. Traditionally, most MRSA isolates were hospital-associated MRSA (HA-MRSA), but more recently, virulent community-associated MRSA (CA-MRSA) strains have become predominant.[22–25] Various genotypes, called pulsed-field types (PFTs), of MRSA have been categorized by the Centers for Disease Control and Prevention.[26] USA300 and USA400 PFTs are associated with CA-MRSA and occasionally USA1100. HA-MRSA strains are typically USA100, USA200, USA500, USA600, and USA800 PFTs. *S. aureus* resistance genes carried mobile genetic elements known as staphylococcal cassette chromosome *mec* (SCC*mec*). CA-MRSA typically carries SCC*mec* IV, V, or VII elements, whereas HA-MRSA typically carries the larger SCC*mec* I, II, III, or VI elements.[26,27] Panton-Valentine leukocidin (PVL) is present in many CA-MRSA isolates and has been shown to be cytotoxic to human monocytes, macrophages, and polymorphonuclear leukocytes.[28,29] An arginine catabolic mobile element (ACME), which inhibits polymorphonuclear cell production, may also be associated with virulence and is typically found in

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community-acquired USA300 PFT strains.[28,30] An increasing level of complexity has been introduced into the characterization of MRSA as CA-MRSA is increasingly being recognized as occurring in hospitalized patients. Therefore, using the traditional criteria of onset in the community versus onset in the hospital does not always reflect PFT or antimicrobial resistance patterns, requiring a combination of all three descriptions to adequately characterize the pathogen's epidemiology.

This changing epidemiology of MRSA is associated with an increased rate of skin and soft-tissue infections (SSTIs), especially among US military recruits and other populations such as athletes and prisoners.[24,31] In 2008, cellulitis and abscess accounted for 63,501 ambulatory visits and more than 1,350 hospital admissions for males and was the seventh most common cause of admission in US military personnel.[32,33] Skin disorders have been a common medical problem in previous wars; and over the past decade, MRSA SSTIs have resulted in increased morbidity and mortality.[34–37] A recently published study from Iraq identified 66 patients with a diagnosis of furuncle or carbuncle, or skin abscess or cellulitis. Of those cultured, 26 had *S. aureus* (MRSA in 15 of these).[38] On the basis of these results, the authors approximated an SSTI rate of 880 cases per 100,000 persons per year and CA-MRSA rate of 600 cases per 100,000 persons per year in US troops in Iraq.

Our study was designed to provide specific genotypic and phenotypic characteristics of MRSA wound culture isolates, including those causing SSTI from a CSH in Iraq. These results provide theater-specific epidemiologic data of types of MRSA strains present, which can be used to enhance the care of patients managed within a combat zone.

## MATERIALS AND METHODS

### Study Location

After approval by the Brooke Army Medical Center Institutional Review Board, MRSA isolates recovered from wound cultures at a CSH in Iraq from December 2007 through March 2009 were collected and stored frozen until they were shipped in batches for further analysis. The CSH is a tertiary care military healthcare facility established in Baghdad, Iraq, for the treatment of US, coalition, and host nation patients. Details of the facility infrastructure have been described previously.[16] Single-patient isolates were recovered from inpatients and outpatients being managed at the facility. In addition, isolates were available from patients managed in a surrounding forward operating base (FOB) medical facility that referred isolates to the CSH microbiology section for pathogen identification and antimicrobial susceptibility testing.

The intheater microbiological system initially characterizing isolates was the MicroScan Autoscan 4 system (Dade Behring, West Sacramento, CA). Isolates were shipped periodically to Brooke Army Medical Center for further characterization. All isolates were stabbed into nutrient agar deeps and packed and shipped in accordance with international and federal regulations. All isolates were imported under permit from the Centers for Disease Control and Prevention. Once the isolates arrived at our facility, the bacteria from them were recovered and placed in freezer beads (Pro-Lab Diagnostics Microbank, Pro-Lab Diagnostics, Austin, TX, http://www.pro-lab.com/products-microbank.php) and stored at −70°C. Although most isolates did not have relevant clinical data, a subset of 17 patients with isolates collected between November 7, 2008, and March 5, 2009, had the referring ward or facility information available. Nationality, anatomic source of isolate, and mechanism of injury were not available for any isolate.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing at our facility was performed using broth microdilution for vancomycin alone to ensure whether vancomycin intermediately resistant isolates were detected as described previously and the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) for the remaining antimicrobials following standardized protocols.[39] In addition, D-zone testing was performed for evaluation of inducible clindamycin resistance on all isolates.[40] Susceptibility was defined based on the approved standards for broth microdilution or the automated system.

### PFGE and Molecular Assessment of Virulence and Resistance

Isolates obtained during this study were assessed by pulsed-field gel electrophoresis (PFGE) after *SmaI* restriction enzyme digestion as described previously.[13,39] PFGE gels were interpreted and grouped into PFT using established criteria.[13,26,39]

A multiplex polymerase chain reaction to detect *mec* element types was performed as described previously (control strains obtained from Hermina de Lencastre).[27,39] This multiplex SCC*mec* polymerase chain reaction included eight loci and the *mecA* gene as an internal control and is able to detect SCC*mec* I, II, III, and IV. The PVL gene was detected as described previously as the *arcA* gene, which is a surrogate marker for type I ACME.[40]

## RESULTS

### Antimicrobial Susceptibility Testing

There were 84 MRSA wound isolates available for analysis; however, it was not possible to differentiate combat-related wounds from SSTIs with associated abscesses. The only antimicrobial agent active against all isolates was vancomycin (Table 1). The most active oral antistaphylococcal agents were tetracycline (95.2% of isolates susceptible) and trimethoprim-sulfamethoxazole (trim-sulf; 94.0% of isolates susceptible). Clindamycin susceptibility was 94.0%. If D-zone testing was incorporated, then there was an overall 87% clindamycin susceptibility. The PFTs associated with inducible clindamycin resistance included type 2 and type E; none of the traditional CA-MRSA PFT USA300 showed inducible clindamycin resistance. Testing of agents not typically used in monotherapy (because of concern of the spontaneous development of resistance) revealed 97.6% of isolates susceptible to rifampin, 90.5% to moxifloxacin, and 59.5% to levofloxacin. Susceptibility to other less commonly used agents included linezolid (97.6%) and daptomycin (96.4%).

**TABLE 1.** Percentage of MRSA Isolates Susceptible to Various Antimicrobial Agents by Pulsed-Field Types

| Pulsed-Field Type (n) | Daptomycin | Erythromycin | Levofloxacin | Linezolid | Moxifloxacin | Quinupristin-Dalfopristin | Rifampin | Tetracycline | Trimethoprim-Sulfamethoxazole | Vancomycin | Clinda | Clindamycin (Inducible Resistance) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total (84) | 81 (96) | 12 (14) | 50 (60) | 82 (98) | 76 (91) | 81 (96) | 82 (98) | 80 (95) | 79 (94) | 84 (100) | 79 (94) | 73 (87) |
| USA300 (66) | 65 (99) | 5 (8) | 39 (59) | 64 (97) | 63 (96) | 64 (97) | 66 (100) | 65 (99) | 64 (97) | 66 (100) | 64 (97) | 64 (97) |
| USA1100 (4) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) | 4 (100) |
| Type 2 (5) | 5 (100) | 0 (0) | 0 (0) | 5 (100) | 0 (0) | 5 (100) | 5 (100) | 5 (100) | 5 (100) | 5 (100) | 5 (100) | 0 (0) |
| Type A (2) | 2 (100) | 0 (0) | 2 (100) | 2 (100) | 2 (100) | 1 (50) | 0 (0) | 0 (0) | 0 (0) | 2 (100) | 0 (0) | N/A |
| Type B (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| Type C (1) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| Type D (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| Type E (1) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 1 (100) | 0 (0) |
| Type F (1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 100 |
| Type G (1) | 1 (100) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | N/A |
| Type H (1) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |

N/A, not applicable.
Data are presented as n (%).

**TABLE 2.** Molecular Characteristics of Isolates, Including Pulsed-Field Types, SCC*mec* Resistance Genes, and ACME and PVL Virulence Genes

| Pulsed-Field Types (n) | SCC*mec*, n (%) | Presence of ACME, n (%) | Presence of PVL, n (%) |
|---|---|---|---|
| USA300 (66) | IV 66 (100) | 62 (94) | 66 (100) |
| USA1100 (4) | IV 2 (50), NA 2 (50) | 0 (0) | 4 (100) |
| Type 2 (5) | II 5 (100) | 0 (0) | 0 (0) |
| Type A (2) | IIIA 1 (50), IIIB 1 (50) | 0 (0) | 0 (0) |
| Type B (1) | IV 1 (100) | 0 (0) | 0 (0) |
| Type C (1) | IV 1 (100) | 0 (0) | 0 (0) |
| Type D (1) | IV 1 (100) | 0 (0) | 1 (100) |
| Type E (1) | IA 1 (100) | 0 (0) | 1 (100) |
| Type F (1) | IV 1 (100) | 0 (0) | 1 (100) |
| Type G (1) | IV 1 (100) | 1 (100) | 1 (100) |
| Type H (1) | IV 1 (100) | 0 (0) | 0 (0) |

Of oral antimicrobial agents, which might be used against PFT USA300 (responsible for most MRSA SSTIs), tetracycline was most commonly active (98.5% susceptibility), followed by trim-sulf (97.0%) and clindamycin (97.0%).

## PFGE and Molecular Assessment of Virulence and Resistance

The most common PFTs were USA300 (78.6%), followed by Type 2 (6.0%) and USA1100 (4.7%; Table 2). The *SCCmec* elements carrying the CA-MRSA resistance genes (SCC*mec* IV) were present in 88.0% of isolates, and the genes associated with HA-MRSA (I, II, and III) were present in 9.5% of the isolates (Table 2). The virulence factor PVL was found in 88.1% of isolates, including 100% of PFT USA300 (Table 2). The ACME virulence factor was present in 75.0% of isolates, including 94.0% of PFT USA300 (Table 2).

## Subpopulation Analysis

Seventeen MRSA isolated obtained between November 7, 2008, and March 5, 2009 had limited clinical data. From the 17 patients, 12 of the isolates were obtained in the outpatient setting, of which 5 were referred from outlining FOBs. The remaining five samples were from patients admitted to the hospital: three from the ward, one from the intensive care unit, and one from the operating room. Of the isolates, 15 were USA300, 1 was type G from a patient's thigh sample collected in the intensive care unit, and one was type H from a skin sample obtained at an FOB. The isolates were 100% susceptible to vancomycin, tetracycline, rifampin, linezolid, moxifloxacin, trim-sulf, and quinupristin-dalfopristin. For this subpopulation only, no isolates were susceptible to erythromycin, 52.9% were susceptible to levofloxacin, 88.2% to daptomycin (resistance was seen in one PFT USA300 and one type H), and 94.1% to clindamycin (resistance in one type G).

# DISCUSSION

*Staphylococcus aureus* infections of combat-related injuries have been described since World War I. However, since the 1990s, new CA-MRSA strains have been increas-

ingly described. CA-MRSA has been recognized as a common pathogen of SSTIs in healthy military personnel during training and while deployed.[24,38] In addition, CA-MRSA infects combat-related injury wounds at the time of injury and also results in chronic complications of extremity injuries, including osteomyelitis.[13,15,17] This study augments the growing knowledge of the impact of MRSA on military service members in and out of a combat zone, providing new genotypic and phenotypic characterization of isolates, which could influence future management strategies for military personnel.[41]

Infections with CA-MRSA USA300 PFT with the virulence factors PVL are commonly encountered in SSTIs associated with abscesses or furuncles in the United States. Data indicate that the ideal therapy of these infections is adequate surgical debridement, with antimicrobial therapy only augmenting care.[42–44] Based on this study, oral therapy with trim-sulf might be the best therapy. A caveat to the use of trim-sulf in SSTIs is its limited activity against the *Streptococcus* species, which is frequently a pathogen causing cellulitis.[45] For adequate coverage of both *Staphylococcus* and *Streptococcus*, tetracyclines might be an ideal alternative antimicrobial agent. Given our routine use of doxycycline for malaria chemoprophylaxis, studies should be undertaken to evaluate the impact of this agent on preventing the development of SSTI. In addition further studies are needed to assess its impact on resistance secondary to ongoing drug pressure, especially with the reports of doxycycline inducing CA-MRSA 300 PFT tetracycline resistance.[46,47] Clindamycin might also be an excellent alternative; however, the clinical impact of inducible resistance that decreased the susceptibility rate from 94% to 87% in this study is unclear.[40] It is noteworthy that this inducible resistance was not found in any isolates with the resistance genes *SCCmec* IV, including the USA300 PFT. It is unclear what impact this inducible resistance has in general on clinical outcomes or why it is not present in these strains. Linezolid might also be an alternative agent; however, it is costly and is associated with notable hematopoietic complications and substantial drug–drug interactions. Because of the spontaneous development of resistance when rifampin or fluoroquinolones are used as monotherapy, these agents are discouraged for routine use in SSTIs or combat-related wound injuries. For those in whom an intravenous therapy is required, vancomycin still seems to be the ideal agent. Given that the primary therapy is adequate surgical management for most MRSA SSTI, even if MRSA is potentially colonizing a wound at the time of combat-related injury, there is no indication for MRSA-directed therapy; and treatment with standard antimicrobials is still recommended.[17,48]

Based on this epidemiologic data and the high rate of possible MRSA SSTIs in theater, an increased emphasis on preventing these infections is needed. A study looking at decolonization with intranasal mupirocin showed limited benefit.[49] Another study assessing periodic cleaning with chlorhexidine towels in marines revealed no significant efficacy (personal communication, Timothy J. Whitman).[50] Overall, improved strategies to prevent infections are needed, and one strategy might include a vaccine that incorporates the virulence genes commonly seen in these strains of bacteria reflective of isolates in our service members.[51,52]

This study has a number of limitations, including the lack of the details pertaining to the clinical care of wounds and prior antimicrobial exposure. It is also unclear whether cultures are from SSTI or are associated with combat-related injuries, which is vital for patient care. It is also necessary to have this information to clearly relate whether the CA-MRSA wound cultures were not only associated with the corresponding typical community PFT and antimicrobial resistance pattern but also onset in the community. Isolates also included those of inpatients and outpatients and are likely reflective of strains from across various levels of care of combat casualties, disease, and nonbattle injuries including battalion aid stations through CSHs. This information is vital because it is unclear how MRSA complicates the care of wounds because it was shown to be present in 2 of 61 wounds at the time of combat-related injury in Iraq but not in wounds a few days after injury in patients treated on the US naval ship Comfort.[17,53] However, MRSA was noted to be associated with osteomyelitis in 8% of combat-related extremity injuries in a level V facility in the United States and in 31% of osteomyelitis recurrences or relapses.[53] These isolates likely reflect the pathogens associated with complications of patients cared for in our military medical healthcare system in and out of the combat zone. Further studies are needed to characterize the wounds in theater and to clearly indicate the type of wound when the pathogen was recovered; combat-related wound versus SSTI with abscess.

Overall, CA-MRSA is increasingly affecting military personnel in and out of the combat zone. The genotypic and phenotypic characterizations of CA-MRSA isolates in theater augments recently characterized epidemiologic studies in the combat zone and in the United States. This study will not only enable improved care, including selection of appropriate antimicrobial therapy, but also guide future intervention and prevention trials.

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## DISCUSSION

**Dr. Kent E. Kester** (Walter Reed Army Institute of Research, Silver Spring, MD): This paper by Murray, et al.

 

describes detailed microbiologic and genetic analyses of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates recovered from wound cultures obtained in a deployed U.S. Army combat support hospital (CSH) in Iraq. Using a variety of standard microbiologic techniques, as well as common genotypic analyses, an effort was made to characterize for the first time the phenotypic and genotypic characteristics of these bacteria recovered from wound cultures in a CSH deployed to Iraq.

Through the use of standard broth microdilution techniques augmented by D-zone testing for the evaluation of inducible clindamycin resistance, antimicrobial susceptibility was performed on samples sent from the deployed CSH in Iraq to Brooke Army Medical Center in San Antonio, TX. Further, genetic analysis of the isolates was performed via the use of pulsed-field gel electrophoresis (PFGE) as well as PCR-based techniques designed to detect SCC*mec* genes associated with antimicrobial resistance, as well as the Panton-Valentine leukocidin (PVL) and the arginine catabolic mobile element (ACME), both of which may be associated with enhanced virulence in selected infections. Of 84 MRSA wound isolates, none were resistant to vancomycin and the antimicrobial susceptibility patterns identified were typical of CA-MRSA. The most common pulsed-field type (PFT) was the USA300 strain, which accounted for nearly 80% of all isolates assessed. Genetic analyses demonstrated that the vast majority of the isolates carried typical SCC*mcc* elements, along with the PVL and ACME virulence factors.

The emergence of MRSA and in particular, CA-MRSA, continues to challenge clinicians, especially since many of these infections are no longer nosocomial in nature, although there is emerging data that suggests that CA-MRSA strains are replacing the previously more common hospital-acquired (HA) strains of MRSA (1-3) in hospitalized patients. Further, in what can only be described as an epidemic, the rates of CA-MRSA isolation and infection in the non-hospitalized civilian population continue to climb (4). While many of these infections are typical staphylococcal skin and soft tissue infections, there are increasing numbers of reports that associated the USA300 genotype of MRSA with necrotizing community-acquired pneumonia (5). The military population has not been left out of this developing epidemic. Dufresne et al., in a retrospective study that evaluated the prevalence of CA-MRSA in soft tissue abscesses in two military level-1 trauma centers, found that CA-MRSA was associated with nearly two-thirds of all soft tissue abscesses in patients who presented to the Emergency Departments of either Brooke Army Medical Center or Wilford Hall Medical Center in San Antonio (6). Roberts et al. retrospectively evaluated MRSA infections identified over a 5-month period at a level 2 treatment facility in military personnel deployed to Iraq and found that approximately 70% of the isolates were MRSA, again demonstrating the increasing impact these organisms have on the etiology of a relatively-common community-acquired infection (7).

As the authors of this paper correctly note, the current report is limited by a lack of clinical data, especially since the presence of organisms in a wound may not necessarily correlate with a true infection. It would be useful to know if the MRSA isolates were collected sequentially (i.e., all isolates collected over the time period of the study submitted for detailed analysis). In the course of the full microbiologic characterization of the isolates, were there any discrepancies noted when the antibiograms were compared with the clinical microbiologic data derived from the referring deployed microbiology laboratory? The data presented in this paper suggests that the use of trimethoprim/sulfamethoxazole would be an appropriate first-line therapeutic choice, although the use of doxycycline is also suggested as an alternative. Whether or not the latter may cause more problems over the longer term related to its own induction of resistance in USA300 isolates remains to be seen (8). Finally, while not a part of this study, it would be useful if there was some type of environmental and/or staff assessment in order to identify potential point sources of certain of the CA-MRSA infections—a very useful tool for improving overall infection control practices in a deployed medical care setting.

This latest study is a natural follow-on to the earlier studies that evaluated MRSA infections in military personnel. The data provided make a compelling case for further study of this new epidemic in military medical treatment facilities, both in inpatients and in outpatients, since CA-MRSA infections are now commonly seen in both populations. The power of the military healthcare system is that as a unified system with common data reporting systems, the impact of CA-MRSA can be assessed system-wide, ideally in the context of a prospective study of all CA-MRSA infections, not just isolated wound cultures. And an important aspect of such a study would include complete clinical information related to the patient, the wound, mechanism of injury (if any), and any other relevant data. A particularly important area for future investigation involves ongoing assessments of the antibiotic susceptibility patterns of these isolates over time, both from an individual facility standpoint, as well as from the entire military healthcare system, since changes in these patterns may have a significant impact on treatment and/or preventive strategies. As described above, environmental and even medical staff assessments may be extremely useful in terms of localizing potential sources of infection. While it is too early to tell if an effective vaccine against CA-MRSA is achievable, the concept certainly merits close attention, since these infections transcend the entire spectrum of healthcare, military and civilian.

The authors are to be congratulated for their efforts to begin to study detailed characteristics of the developing CA-MRSA epidemic. Studies of this type as well as the natural expanded follow-on studies will not only assist in the better definition of multiple characteristics of CA-MRSA and associated infections, but will also materially contribute to the long-term improvement of the medical care of military patients.

**Dr. Clinton Murray** (Brooke Army Medical Center, Fort Sam Houston, TX): We appreciate Dr. Kester's kind words and support of this project and have attempted to

address his relevant issues. The isolates evaluated in this study were serial isolates collected and stored in the CSH's clinical microbiology section. However, the isolates referred from outside of the facility may not necessarily be serial for those facilities. There did not appear to be discrepancies between the testing method in theater for antimicrobial resistance testing and those test performed at BAMC; however, some testing, such as D-zone and broth microdilution, were not performed in the theater clinical microbiology section. We do agree that follow on studies are needed of environmental isolates along with greater clarity of type of infections, timing of infection, outpatient versus inpatient onset of infection, prior antimicrobial exposure and other clinically relevant information to best inform the clinician on how to adequately manage combat-related wound infections and skin and soft tissue infections.